(32) M. M. Campos and H. Hauptmann, J. Am. Chem. Soc., 74, 2962 (1952).
(33) G. W. H. Cheeseman, J. Chem. Soc., 458 (1959).
(34) W. von E. Doering and L. K. Levy, J. Am. Chem. Soc., 77, 509 (1955).
(35) E. Fromm, Justus Liebigs Ann. Chem., 253, 135 (1889).
(36) H. J. Backer, Recl. Trav. Chim. Pays-Bas, 71, 409 (1952).
(37) C. K. Ingold and J. A. Jessop, J. Chem. Soc., 2359 (1929).
(38) E. Steger and I. Lorenz, J. Prakt. Chem., 13, 272 (1961).
(39) R. Stroh, R. Seydel, and W. Hahn in "Newer Methods of Preparative Organic Chemistry," Vol. II, W. Foerst, Ed., Academic Press, New York, N.Y., 1963, p 354.
(40) J. B. Hendrickson, A. Giga, and J. Wareing, J. Am. Chem. Soc., 96, 2275 (1974).
(41) H. Böhme, H. Fischer, and R. Frank, Justus Liebigs Ann. Chem., 563, 54 (1949).
(42) T. Mukaiyama, S. Kobayashi, and T. Kumamoto, Tetrahedron Lett., 5115 (1970).
(43) J. E. Dunbar and J. H. Rodgers, J. Org. Chem., 31, 2842 (1966).
(44) D. S. Noyce and R. M. Pollack, J. Am. Chem. Soc., 91, 119 (1969).
(45) O. Behaghel and H. Seibert, Ber., 66, 708 (1933).
(46) N. D. Epiotis, R. L. Yates, F. Bernardi, and S. Wolfe, J. Am. Chem. Soc., 98, 5435 (1976).
(47) J.-M. Lehn and G. Wipff, J. Am. Chem. Soc., 98, 7498 (1976).

Rates of Thiol–Disulfide Interchange Reactions between Mono- and Dithiols and Ellman's Reagent[1]

George M. Whitesides,* Jennifer E. Lilburn, and Richard P. Szajewski

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

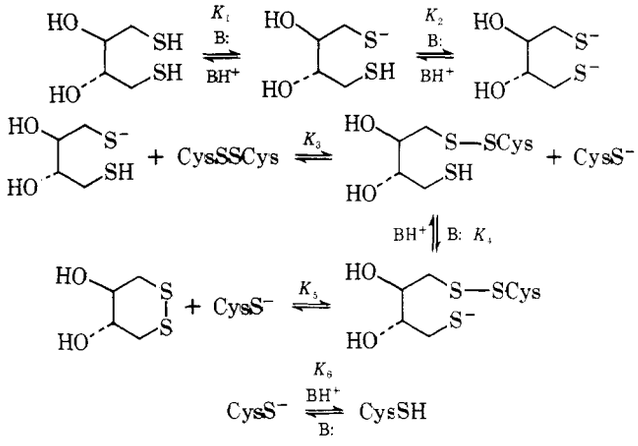
Received July 14, 1976

The rate constants for thiol–disulfide interchange between 21 mono- and dithiols and Ellman's reagent correlate with the pK_a's of the thiol groups with a Bronsted coefficient of $\beta = 0.36$. The maximum rates of reduction are observed for thiols having pK_a values close to the pH of the solution in which the reactions were carried out. In the dilute solutions examined (10^{-4}–10^{-6} M in each reagent), the rate of the second, intramolecular interchange step in reactions of dithiols was faster than that of the first, intermolecular interchange, regardless of the size of the cyclic disulfide formed. A convenient synthesis of a mixture of diastereomers of 1,4-dimercapto-2,3-butanediol (i.e., of a mixture of dithiothreitol, DTT, and dithioerythritol, DTE) has been developed from 1,2:,4-diepoxybutane and thiolacetic acid.

Oxidation of cysteine sulfhydryl groups during isolation, storage, and use of proteins is often an important contributor to their deactivation.[2] Although the rate of oxidation can be decreased by limiting access of oxygen to the enzyme, it is usually impractical to exclude oxygen completely, particularly in practical synthetic and analytical applications. The most effective and widely used reagents for protecting the cysteine moieties of enzymes against oxidation by adventitious oxygen, and for activating partially oxidized and deactivated enzymes by reduction, are thiols, particularly dithiothreitol (DTT, Cleland's reagent)[3] and β-mercaptoethanol. Each has its advantages and disadvantages: DTT reduces protein disulfide groups rapidly and completely and is convenient to handle, but is exorbitantly expensive; β-mercaptoethanol is readily available and inexpensive, but reacts less rapidly and completely.

As part of a project designed to develop techniques to permit the use of enzymes as catalysts in large-scale organic synthesis, we required an agent that would reduce disulfide moieties more rapidly and completely than β-mercaptoethanol but which would be less expensive than DTT. The design of an appropriate reagent is not straightforward for several reasons. First, the mechanism of reduction (illustrated in Scheme I for DTT) involves multiple acid–base and sulfhydryl–disulfide interchange equilibria, and the dependence of the overall rate and equilibrium position on the structure of the reducing agent (and possibly of the protein) is difficult to predict. An important part of the difference in reactivity between DTT and β-mercaptoethanol can, however, plausibly be attributed to the rate of release of the second equivalent of $CysS^-$ (or CysSH) from initially formed mixed disulfides: since β-mercaptoethanol is commonly used in enzymology at concentrations of ca. 10 mM, the rate of the intermolecular reaction involved in its release of CysSH from $CysSSCH_2CH_2OH$ should be approximately 10^{-3}–10^{-4} the rate of the corresponding intramolecular release from $CysSSCH_2CHOHCHOHCH_2SH$. Second, a useful reducing reagent, in addition to high reactivity and ready availability, should also have good water solubility, tolerable odor, and low toxicity. These requirements seriously limit the range of possible thiols.

Scheme I. Mechanism of Reduction of a Cystine Moiety CysS–SCys by DTT



Here we describe an examination of the rates of reaction of a number of mono- and dithiols with 5,5′-dithiobis(2-nitrobenzoic acid) (Ellman's reagent, EllS-SEll).[4] This study represents the first phase of an effort to understand the kinetics and equilibria of biochemically relevant thiol–disulfide interchange reactions in sufficient detail to be able to rationalize the exceptionally useful properties of DTT in terms of its structure, and to design alternative, effective reducing agents. Ellman's reagent was chosen as the disulfide for initial examination for several reasons. First, since the S–S bond is weak, its reduction by most thiols should be complete: it should thus be possible to examine the influence of the structure of a reducing thiol on its rate of reaction with the disulfide bond of Ellman's reagent without complications by

a competing reverse reaction. Second, the reduction of Ellman's reagent is easily followed spectrophotometrically. Third, Ellman's reagent is a reagent widely used for the determination of sulfhydryl groups, and information concerning the rates of its reduction by thiols should be useful in these other applications.

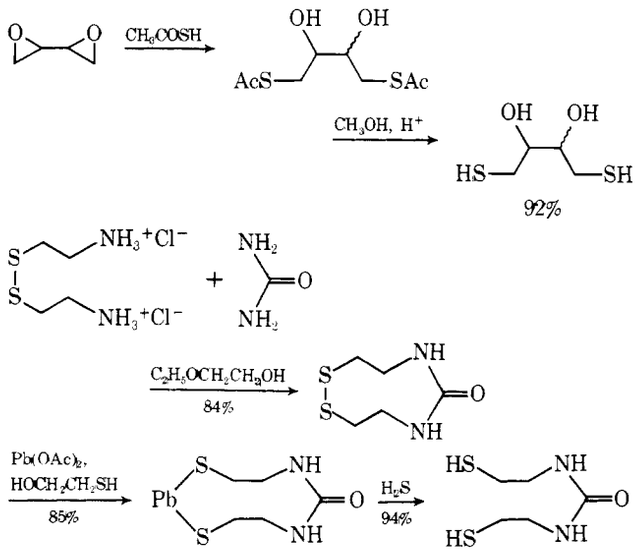
Although data obtained from these studies cannot be applied directly to the design of reagents for the reduction of protein disulfide moieties, they should provide answers to two fundamental questions that underlie this problem: Do the rates of reduction of a particular disulfide moiety by thiol reducing agents and the pK_a's of the SH groups of these reducing agents obey a Bronsted relation? What is the optimum pK_a for the thiol group of a reducing agent intended to be used at a particular solution pH?

Results

Synthesis of Sulfhydryl Reducing Agents. All of the characteristics of DTT as a reagent for reducing disulfide moieties are satisfactory except for its expense. A solution to the problem of providing an effective, inexpensive reducing agent could come either from a reduction in the cost of DTT, or by developing an alternative, easily prepared, material having comparable properties.

DTT is prepared from 1,4-dibromo-2-butene by a four-step stereospecific synthesis.[5] Since DTT and DTE have similar activity, and since their enantiomeric and diastereomeric purity is almost certainly irrelevant in most applications, this synthesis is unnecessarily complicated. It proved possible to prepare mixtures of diastereomeric 1,4-dimercapto-2,3-butanediols in excellent yield by reaction of 1,2:3,4-diepoxybutane with thiolacetic acid[6] followed by acid-catalyzed deacylation (transesterification) in methanol (Scheme II). The

Scheme II. Synthesis of 1,4-Dimercapto-2,3-butanediol and *N,N'*-Bis(2-mercaptoethyl)urea

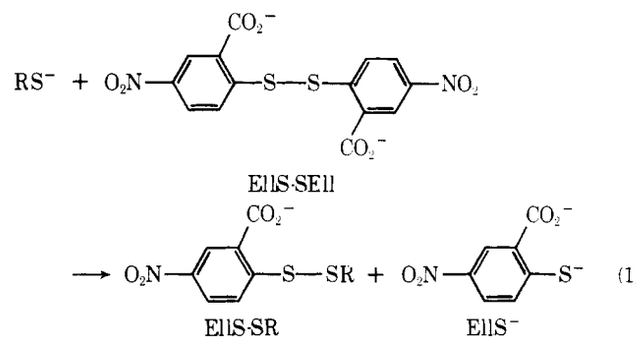


direction of the acid-catalyzed epoxide opening is presumably controlled by the inductive effect of the adjacent oxygens.[7] A preparation of the same dithiol from hydrogen sulfide and diepoxybutane is reported in the patent literature.[8] Diepoxybutane is prepared by oxidation of butadiene,[9] and its generation and utilization in situ should decrease the problems of toxicity and storage stability associated with the pure compound.

Preparation of thiols and disulfides from halides and tosylates is often cumbersome: commonly used procedures have been reviewed[10] and most appear impractical as the basis for large-scale synthesis. We have examined several routes based on condensation reactions: one sequence, leading from cystamine hydrochloride and urea to *N,N'*-bis(2-mercaptoethyl)urea, is outlined in Scheme II. In this and related efforts, it proved most convenient for small-scale work initially to prepare and purify disulfides and subsequently to reduce these to the desired dithiols, rather than toprepare the dithiols directly.

Measurements of Rates of Reduction of Ellman's Reagent by Thiols. Rates were obtained at 30.0 °C and pH 7.0 (0.05 M phosphate buffer) in oxygen-free solutions containing Ellman's reagent and (di)thiol by following the absorbance with time at 412 nm, the λ_{max} for the anion of 2-nitro-5-thiobenzoic acid (Ellman's anion); Ellman's reagent itself does not absorb significantly at this wavelength. At pH 7.0, the generation of Ellman's anion by reaction of Ellman's reagent with hydroxide ion[11] was insignificant compared with its production by reaction with thiol. Control experiments established that low concentrations of added copper(II) salts had no influence on the measured rates, provided that oxygen was excluded. As expected, copper(II) was an active catalyst for oxidation by air.[12] Irradiation with uv light also had no influence on rates. The thiol–disulfide interchange appears to be influenced by buffer concentration and composition: all reactions were therefore carried out using a standard buffer and concentration (0.05 M phosphate).

The kinetic schemes used as the basis for analyzing the reduction of Ellman's reagent by mono- and dithiols are defined in the following equations.



(1)

For monothiols

$$RSH \overset{K_a}{\rightleftharpoons} RS^- + H^+ \quad (2)$$

$$RS^- + EllS\text{-}SEll \xrightarrow{k_1} RS\text{-}SEll + EllS^- \quad (3)$$

$$RS^- + RS\text{-}SEll \xrightarrow{k_2} RS\text{-}SR + EllS^- \quad (4)$$

$$d(EllS^-)/dt = k_1^{obsd}[(RS^-) + (RSH)](EllS\text{-}SEll) + k_2^{obsd}[(RS^-) + (RSH)](EllS\text{-}SR) \quad (5)$$

Here k_1^{obsd} and k_2^{obsd} are observed rate constants based on an experimental rate equation (5) containing terms in the total concentration of sulfhydryl species $[(RS^-) + (RSH)]$, and k_1 and k_2 are rate constants for reactions involving the thiolate anions (reactions 3 and 4). This problem requires analysis of competitive, consecutive, second-order reactions.[13] Preliminary analysis showed that $k_1 > 10k_2$. It was possible to take advantage of this difference in rate constants to separate the terms in k_1^{obsd} and k_2^{obsd} in eq 5 by fixing the relative concentrations of reducing agent and Ellman's reagent so that the contribution of either reaction 3 or reaction 4 to the production of $EllS^-$ was negligible. Thus, to obtain k_1^{obsd}, the initial concentration of thiol reagent was set at any value less than one-half that of Ellman's reagent (eq 6).

$$2[(RS^-) + (RSH)]_0 \leq (EllS\text{-}SEll)_0 \quad (6)$$

Table I. Rate Constants (k, M^{-1} s^{-1}) for Reduction of Ellman's Reagent by Mono- and Dithiols[a]

Registry no.		Thiol	pK_a	k_1^{obsd}	k_2^{obsd}	k_1	k_2	Solubility[b]
98-91-9	1	C_6H_5COSH	2.48[c]	1.0×10^2	39.4	1.0×10^2	39.4	$\leq 10^{-2}$
507-09-5	2	CH_3COSH	3.5[d]	2.0×10^2	82.3	2.0×10^2	82.3	>1
3814-18-4	3	m-$NO_2C_6H_4SH$[e]	5.24[f]	1.4×10^4	g	1.5×10^4	g	$\leq 10^{-2}$
1074-36-8	4	p-$HO_2CC_6H_4SH$[h]	5.80[i]	1.3×10^4	g	1.3×10^4	g	$\leq 10^{-2}$
106-53-6	5	p-BrC_6H_4SH	6.02[f]	3.1×10^4	g	3.5×10^4	g	$\leq 10^{-2}$
15570-12-4	6	m-$CH_3OC_6H_4SH$	6.39[f]	4.1×10^4	g	5.1×10^4	g	$\leq 10^{-2}$
137-07-5	7	o-$NH_2C_6H_4SH$	6.59[j]	3.3×10^4	g	4.6×10^4	g	$\leq 10^{-2}$
108-98-5	8	C_6H_5SH	6.62[f]	1.6×10^4	g	2.6×10^4	g	$\leq 10^{-3}$
123-81-9	9	$(HSCH_2CO_2CH_2)_2$	7.70 (8.97)	8.8×10^3	k	5.2×10^4	k	$\leq 10^{-2}$
760-30-5	10	$H_2NNHCOCH_2SH$	7.75	7.8×10^3	g	5.1×10^4	g	$\sim 10^{-1}$
59-52-9	11	$HOCH_2CHSHCH_2SH$	8.59 (10.5)	2.7×10^3	k	1.1×10^5	k	≥ 1
584-04-3	12	$HSCH_2CHOHCH_2SH$	9.04 (10.3)	2.6×10^3	k	2.8×10^5	k	≥ 1
3570-55-6	13	$(HSCH_2CH_2)_2S$	9.09 (10.1)	2.5×10^3	k	3.1×10^5	k	$\leq 10^{-3}$
6892-68-8	14	DTT	9.21 (10.1)	2.5×10^3	k	2.9×10^5	k	≥ 1
3483-12-3	15	DTT/DTE[l]	9.2 (10.1)	2.3×10^3	k	2.7×10^5	k	≥ 1
60633-86-5	16	$(HSCH_2CH_2NH)_2CO$	9.26 (10.0)	2.1×10^3	k	2.5×10^5	k	$\leq 5 \times 10^{-3}$
2150-02-9	17	$(HSCH_2CH_2)_2O$	9.21 (9.91)	5.4×10^2	k	8.7×10^4	k	$\leq 10^{-2}$
60-24-2	18	$HOCH_2CH_2SH$[m]	9.5[n]	6.2×10^2	28	2.0×10^5	8.7×10^3	≥ 1
96-27-5	19	$HOCH_2CHOHCH_2SH$	9.5[d]	1.0×10^3	20	3.2×10^5	6.4×10^3	≥ 1
68-11-1	20	$HSCH_2CO_2H$	9.8[n]	3.0×10^2	2.7	1.8×10^5	2.1×10^3	≥ 1
1191-08-8	21	$HS(CH_2)_4SH$	9.98 (10.7)	9.4×10^2	k	9.0×10^5	k	$\leq 10^{-3}$

[a] All rates were obtained in 0.05 M phosphate buffer, pH 7.0, 30.0 °C containing 10^{-6} M EDTA under an argon atmosphere. Rate constants have the units M^{-1} s^{-1}. [b] Approximate limiting solubility (M) in this buffer system. [c] J. Hipkin and D. P. N. Satchell, *Tetrahedron*, **21**, 835 (1965). [d] J. P. Danehy and K. N. Parameswaran, *J. Chem. Eng. Data*, **13**, 386 (1968). [e] Prepared by reduction [C. R. Stahl and S. Siggia, *Anal. Chem.*, **29**, 154 (1957)] of the corresponding disulfide (W. A. Sheppard, "Organic Syntheses", Collect. Vol. V, Wiley, New York, N.Y., 1973, p 843). [f] P. DeMaria, A. Fini, and F. M. Hall, *J. Chem. Soc., Perkin Trans. 2*, 1969 (1973). [g] Not determined. [h] L. Nelander, *Acta Chem. Scand.*, **18**, 973 (1964). [i] R. J. Irving, L. Nelander, and I. Wadso, *ibid.*, **18**, 769 (1964). [j] M. R. Crampton, *J. Chem. Soc. B*, 2112 (1971). [k] The rate of reaction 15 (text) was greater than that of reaction 13: k_2^{obsd} and k_2 could not be determined. [l] A mixture of diastereomers prepared from diepoxybutane. [m] In 0.05 M HEPES buffer (pH 6.9), $k_1^{obsd} = 2.2 \times 10^2$ M^{-1} s^{-1}; in 0.05 M TEA buffer (pH 6.9), $k_1^{obsd} = 3.0 \times 10^2$ M^{-1} s^{-1}. [n] Reference 15.

$$d(EllS^-)/dt \simeq k_1^{obsd}[(RS^-) + (RSH)](EllS\text{-}SEll) \quad (7)$$

Under these conditions, reaction 4 does not contribute significantly to the production of $EllS^-$, and the rate expression 5 can be approximated by the simplified expression of eq 7. Similarly, by setting the initial concentration of Ellman's reagent to be less than that of thiol (typically a factor of 10: eq 8), production of $EllS^-$ occurs in two kinetically distinct phases: an initial, fast phase corresponding to reaction 3 in which the Ellman's reagent is converted essentially quantitatively to EllS-SR, followed by a slower phase (reaction 4), which can be described by the approximate rate expression 9.

$$[(RS^-) + (RSH)]_0 \geq 10(EllS\text{-}SEll)_0 \quad (8)$$

$$d(EllS^-)/dt \simeq k_2^{obsd}[(RS^-) + (RSH)]_0(EllS\text{-}SR) \quad (9)$$

Equation 7 can be integrated by standard procedures to yield eq 10.[13] Equation 9 is treated similarly.

$$\frac{1}{[(RS^-) + (RSH)]_0 - (EllS\text{-}SEll)_0} \times \ln \frac{(EllS\text{-}SEll)_0}{[(RS^-) + (RSH)]_0}\left[\frac{[(RS^-) + (RSH)]_0 - (EllS^-)}{(EllS\text{-}SEll)_0 - (EllS^-)}\right] = k_1^{obsd}t \quad (10)$$

Since the reactivity of the thiolate anion, RS^-, is so much greater than that of the thiol, RSH, any contribution to the generation of $EllS^-$ from the latter can be neglected. The observed rate constants k^{obsd} and the rate constants for reaction of thiolate anion k (eq 3, 4) can then be related by eq 11a: here pK_a is defined by the acid dissociation of the thiol (eq 2), and pH is that of the solution (in these experiments, pH 7.00).

$$k_1 = k_1^{obsd}[1 + 10^{pK_a - pH}] \quad (11a)$$

$$k_2 = k_2^{obsd}[1 + 10^{pK_a - pH}] \quad (11b)$$

For dithiols

$$HSRSH \overset{K_a}{\rightleftharpoons} HSRS^- + H^+ \quad (12)$$

$$HSRS^- \overset{K'_a}{\rightleftharpoons} {}^-SRS^- + H^+ \quad (12a)$$

$$HSRS^- + EllS\text{-}SEll \xrightarrow{k_1} HSRS\text{-}SEll + EllS^- \quad (13)$$

$${}^-SRS^- + EllS\text{-}SEll \xrightarrow{k'_1} {}^-SRS\text{-}SEll + EllS^- \quad (13a)$$

$$HSRS\text{-}SEll \overset{K''_a}{\rightleftharpoons} {}^-SRS\text{-}SEll \quad (14)$$

$${}^-SRS\text{-}SEll \xrightarrow{k_2} SRS + EllS^- \quad (15)$$

$$HSRS\text{-}SEll + HSRS^- \xrightarrow{k_3} HSRS\text{-}SRSH + EllS^- \quad (16)$$

We simplify this set of equations by noting that these reactions occur in dilute solutions (10^{-4}–10^{-6} M), and then assuming that, under these circumstances, the intermolecular thiol–disulfide interchange involving the mixed disulfide (reaction 16) does not compete with the intramolecular reaction (reaction 15). The correctness of this assumption clearly depends on the relative magnitudes of k_2 and k_3. If the ring formed in reaction 15 were sufficiently strained, this reaction might, in principle, be much slower than reaction 16 even in dilute solution. On the basis of limited precedent, however, it seems unlikely that ring strain in five-, six-, and seven-membered cyclic disulfides would be sufficient to decrease k_2

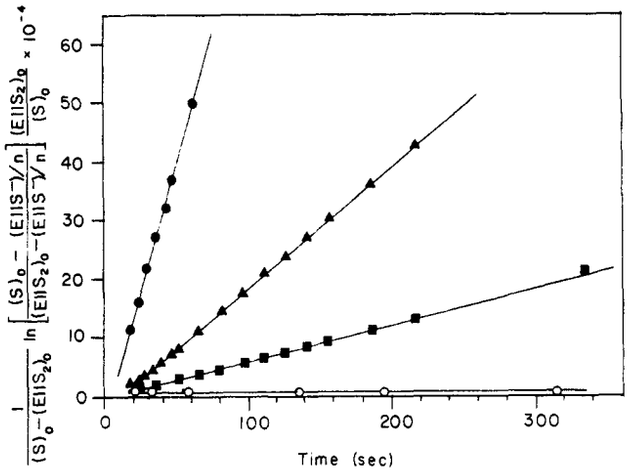


Figure 1. Rate constant plots for the reaction of several mono- and dithiols with Ellman's reagent in pH 7.0 phosphate buffer (0.05 M) at 30.0 ± 0.5 °C under argon. The terms in the expression on the axis are defined as follows: for the monothiols, $S_0 = [(RS^-) + (RSH)]$, $n = 1$; for the dithiols, $S_0 = [(^-SRS^-) + (HSRS^-) + (HSRSH)]$, $n = 2$ (cf. eq 10 and 18 of the text). Reducing agents: ●, glycol dimercaptoacetate; ■, bis(2-mercaptoethyl) ether; ▲; *N,N'*-bis(2-mercaptoethyl)urea; ○, thioglycolic acid.

to the point where the rates of reactions 15 and 16 would be comparable.[14] There is no way of estimating ring strain in large rings containing many heteroatoms [e.g., that from *N,N'*-bis(2-mercaptoethyl)urea], but this strain is almost certainly less than that in saturated carbocycles of the same size.

With these approximations, analysis of the experimental data obtained from solutions of Ellman's reagent and dithiols becomes closely analogous to that for monothiols. A rate equation of the form 17 was followed. In this equation, $(S_{total}) = [(^-SRS^-) + (^-SRSH) + (HSRSH)]$.

$$d(EllS^-)/dt \cong 2k_1^{obsd}(S_{total})(EllS\text{-}SEll) \qquad (17)$$

Straightforward consideration of material balance permits the variable concentrations in the equation to be expressed in terms of $(EllS^-)$ and the resulting expression integrated as eq 18.

$$\frac{1}{(S_{total})_0 - (EllS\text{-}SEll)_0} \times \ln \frac{(EllS\text{-}SEll)_0}{(S_{total})_0} \left[\frac{(S_{total})_0 - \tfrac{1}{2}(EllS^-)}{(EllS\text{-}SEll)_0 - \tfrac{1}{2}(EllS^-)} \right] = k_1^{obsd}t \qquad (18)$$

The factor of 2 in eq 17 reflects the assumption that reaction 13 is rate limiting, and the production of a second equivalent of $EllS^-$ by reaction 15 follows rapidly, once the intermediate mixed disulfide HSRS-SEll is formed. Experimental support for this assumption derives from the observation in reductions using dithiols that there was no suggestion of the two-stage production of $EllS^-$ characteristic of the monothiols, even when the initial concentration of dithiol was much greater than that of Ellman's reagent: the experimental data were compatible with eq 18.

Without examining the change in k_1^{obsd} with pH there is no method of separating this term into the individual rate constants characteristic of the species lumped under the term S_{total}: viz., $^-SRS^-$, ^-SRSH, and HSRSH. To assign a rate constant, we assume, as previously, that the reactivity of the neutral thiol HSRSH is so low compared to that of the thiolates that it can be neglected, and, further, that $^-SRS^-$ and ^-SRSH are equally reactive. The former assumption is undoubtedly good. The latter is to some extent in error: $^-SRS^-$ will be more reactive than ^-SRSH. The dianion will, however, be present in lower concentrations that the monoanion, and their reactivity difference is probably not large. The rate constant k_1 in reaction 13 can then be approximated by an expression analogous to eq 11. Estimates of k_1 in this manner will be too high since they will include a contribution from the (faster) reaction of the dithiolate species $^-SRS^-$: this inaccuracy may contribute to the scatter in the Bronsted plot derived from these data (vide infra). Since, however, the concentration of $^-SRS^-$ is less than that of ^-SRSH, no statistical correction of these rate constants is required.

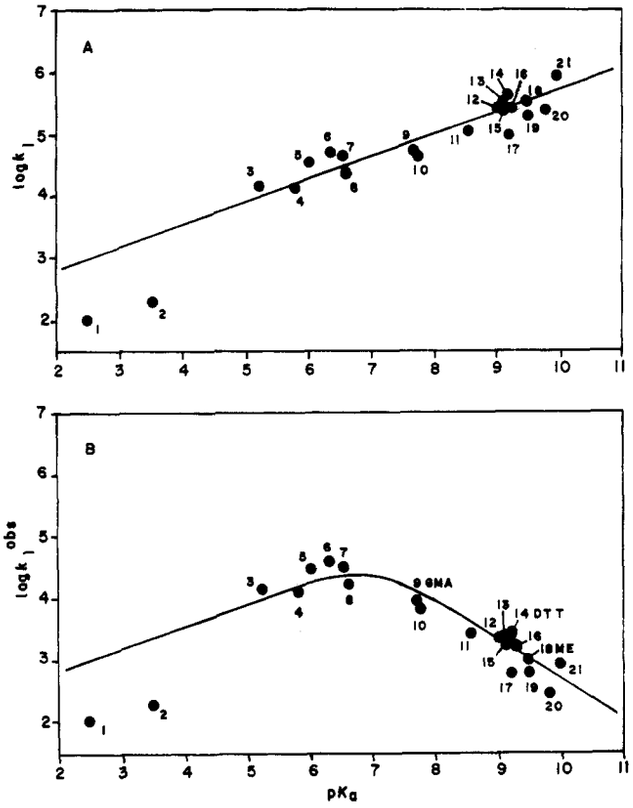


Figure 2. Plots of (A) $\log k_1$ and (B) $\log k_1^{obsd}$ vs. pK_a for reduction of Ellman's reagent in pH 7.0 phosphate buffer (0.05 M) at 30.0 ± 0.5 °C under argon with the mono- and dithiols listed in Table I. The equations used to generate the solid lines are (A) $\log k_1 = 2.1 + 0.36\ pK_a$; (B) $\log k_1^{obsd} = 2.1 + 0.36\ pK_a - \log (10^{pK_a - 7.0} + 1)$. The numbers refer to Table I. DTT is dithiothreitol; ME is β-mercaptoethanol; GMA is glycol dimercaptoacetate. The parameters for the Bronsted line ($\log G = 2.1$, $\beta = 0.36$) were estimated neglecting the thiol acids (points 1 and 2).

Table I summarizes the rate constants derived from kinetic examination of the reduction of Ellman's reagent by various thiols. Three useful facts emerge immediately from analysis of these rate constants. First, the values of k_1 (with the exception of those for thiolacetic and thiobenzoic acid) approximately obey a Bronsted relation, with $\beta = 0.36$ (Figure 2). This value is compatible with values of β found for reactions of thiols with other types of substrates: *N-p*-2-benzimidazolylphenylmaleimide, $\beta = 0.42$;[15] *p*-nitrophenyl acetate, $\beta = 0.38$;[16] ethylene oxide, $\beta = 0.30$;[17] and benzene oxide, $\beta = 0.22$.[18] Second, for the five compounds of Table I for which both k_1 and k_2 were determined, $k_2 \lesssim 0.1k_1$. This conclusion helps to justify the experimental conditions chosen to reduce eq 5 to eq 7 and 9, and to generate eq 17. Third, assuming that the rate of attack of thiol reagent on Ellman's reagent does follow a Bronsted relationship with $\beta = 0.36$, it is possible to derive a relation between thiol pK_a and solution pH that permits a prediction of the pK_a value that will lead to a max-

imum value of k_1^{obsd} at a particular solution pH. By the definition of a Bronsted relation, one can relate k_1^{obsd} to k_1 using eq 19 and 20.

$$k_1 = G10^{\beta pK_a} \quad (19)$$

$$k_1^{obsd} = k_1 \frac{(RS^-)}{(RS^-) + (RSH)} = \frac{G10^{\beta pK_a}}{1 + 10^{pK_a - pH}} \quad (20)$$

Differentiating eq 20 with respect to pK_a, setting $(\partial k_1^{obsd}/\partial pK_a)$ equal to zero, and solving, one obtains eq 21 as the condition for which k_1^{obsd} is a maximum.

$$\frac{1-\beta}{\beta} = 10^{pH-pK_a} \quad (21)$$

Thus, for $\beta = 0.36$, k_1^{obsd} will maximize when $pK_a = pH - 0.25$. Figure 2 plots the values of log k_1^{obsd} and log k_1 from Table I against the thiol pK_a values. This figure also includes a plot of a theoretical curve for log k_1^{obsd} vs. pK_a.

Discussion

The rate constants k_1 characterizing the attack of a number of organic thiolate ions on the disulfide linkage of Ellman's reagent in aqueous solution follow a Bronsted relation: the nucleophilic reactivities of the thiolate ions are directly proportional to their basicity. Since the fraction of a particular thiol present in solution in the reactive thiolate form depends upon the thiol pK_a and the solution pH, k_1 is not, however, the most useful parameter in characterizing the reactivity of a thiol toward Ellman's reagent: the observed rate constants, k_1^{obsd}, provide more direct measures of reactivity. The prediction that k_1^{obsd} for thiols obeying a Bronsted relation with $\beta = 0.36$ should have its maximum value when $pK_a = pH - 0.25$ is supported by experimental data at pH 7 (Figure 2). This figure also indicates that neither of the commonly used reducing agents DTT nor β-mercaptoethanol is the fastest thiol reagent to attack Ellman's reagent in solutions at pH 7: the values of k_1^{obsd} for the various thiophenols listed in Table I are approximately ten times larger than those for DTT and β-mercaptoethanol.

The kinetic behavior of all of the dithiols tested indicates that the initial, intermolecular attack on Ellman's reagent is rate limiting, and that the subsequent, intramolecular step is fast. Four- and five-membered rings (and possibly large rings) containing disulfide linkages are strained. In the dilute solutions studied, however, this strain is evidently not sufficient to cancel the concentration advantage conferred by the intramolecularity of the second reaction.

These results define the influence of thiol structure on the *rate* of its reaction with Ellman's reagent, and suggest two structural features which should be included in new reagents for reduction of cystine disulfide groups. An effective reagent should be a dithiol itself easily capable of forming an intramolecular disulfide, and at least one thiol should have a pK_a close to the pH of the solution in which it is to be used. The properties of DTT are compatible with both of these criteria, although the thiol pK_a values are too high for optimum rates at pH 7.

These data do not in themselves provide an adequate basis for the design of effective reagents for reduction of protein cystine units for two reasons. First, although reduction of Ellman's reagents with the thiols examined here (with the exceptions of the two acidic thio acids) goes to completion, reduction of typical cystine moieties is much less favorable thermodynamically, and the influence of the structure of the dithiol reagent on the equilibrium constant for reduction becomes an important factor. Second, although reduction of Ellman's reagent is a kinetically simple reaction, there is no assurance that electronic factors other than those reflected in a Bronsted plot, or steric or solvation effects, are unimportant in determining the kinetics of reduction of protein cystine groups. In fact, preliminary results in these laboratories indicate no clear correlation between the rates of reduction of Ellman's reagent by thiols and the effectiveness of these substances as reducing agents for proteins.

Experimental Section

General Methods. Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. pH was determined using a Radiometer Model 28 pH meter. Infrared spectra were taken using a Perkin-Elmer Model 567 grating spectrophotometer. NMR spectra were recorded on a Varian T-60 spectrometer. Uv spectra were measured using a Gilford Model 240 spectrometer equipped with a sample chamber thermostated at 30.0 ± 0.5 °C. Mass spectra were recorded on a Hitachi Perkin-Elmer RMU-6E spectrometer at an ionization potential of 20 eV. Elemental analyses were performed by Robertson Laboratory, Florham Park, N.J. Distilled water was passed through an ion exchange column and redistilled using a Corning AG-1B still.

Materials. Thin layer chromatography used J. T. Baker silica gel, grade 1 B. Argon (Airco welding grade) was used without further purification. Unless otherwise stated, pure grade solvents were used without further purification. 1,2:3,4-Diepoxybutane was obtained from ROC/RIC, 2,2′-dithiobis(ethylamine) dihydrochloride from Sigma Chemical Co., and dithiodiglycolic acid (96%), thioglycolic acid (98%), methyl thioglycolate (98%), thiolacetic acid (97%), and 2-mercaptoethanol (98%) were obtained from Aldrich Chemical Co. Glycol dimercaptoacetate was a gift of Evans Chemetics, Darien, Conn.

Thiols were either recrystallized or distilled under vacuum. Ellman's reagent (Aldrich, 99%) was used without further purification. All other chemicals used were AR grade.

1,4-Dimercapto-2,3-butanediol (a Mixture of Dithiothreitol and Dithioerythritol). To 48 g (0.63 mol) of thiolacetic acid at 0 °C under argon was added dropwise, with stirring, 25.8 g (0.30 mol) of 1,2:3,4-diepoxybutane followed by 2 ml of methanol. *Caution. Butadiene diepoxide, like many similar dialkylating agents, is mutagenic. It should be used in a good hood, and care taken to avoid contact with liquid or vapor.* The mixture was allowed to stir at room temperature. Within 48 h a crystalline mass of the epimeric 1,4-dimercaptoacetyl-2,3-butanediols had developed. A sample washed free of residual oils with ether–pentane (1:1) showed mp 64–68 °C; ir (CH_2Cl_2) 3400, 2920, 2890, 1720, 1675 cm^{-1}; NMR ($CDCl_3$) δ 3.8–3.4 (m, 2), 3.3–2.9 (m, 6), 2.40 (s, 6). This crude material, after treatment at reflux under argon with 150 ml of degassed methanol and a catalytic amount of HCl (2 ml of ~1 N HCl in ether) for 6 h followed by sequential distillation of methyl acetate and methanol, gave 42.8 g (92%) of a diastereomeric mixture of 1,4-dimercapto-2,3-butanediols (bp 120–125 °C at 0.1–0.3 mm) which crystallized on standing in the cold: mp 12–15 °C; ir (film) 3450, 2920, 2550 cm^{-1}; NMR ($CDCl_3$) δ 3.9–3.5 (m, 2), 3.3 (s, 2), 2.9–2.5 (m, 4), 1.66 (t, 2, J = 8 Hz). The distilled product is contaminated with 2–5% of 1,3-dimercapto-2,4-butanediols and related materials. The diastereomeric composition of this material was not examined.

2,9-Diaza-5,6-dithiacyclononanone. In a 2-l. round-bottomed single-necked flask were combined 1.5 l. of distilled 2-ethoxyethanol, urea (9.01 g, 0.15 mol), and cystamine hydrochloride (33.7 g, 0.15 mol). The mixture was flushed with argon for 0.5 h, and refluxed under argon for 14 h. The mixture was cooled to room temperature under argon and filtered, and most of the solvent removed under reduced pressure. The product was precipitated on cooling, isolated, and washed with ether. The remaining 2-ethoxyethanol was removed under reduced pressure, leaving a product which was triturated with methanol to remove the brown gum coating it. A yield of 22.3 g (84%) of 1,9-diaza-5,6-dithiacyclononanone was obtained. The product, after recrystallization from methanol, showed mp 207–208 °C; ir (Nujol) 3340, 1625, and 1590 cm^{-1}; NMR (CF_3CO_2H) δ 2.2–2.7 (m, 4), 3.0–3.5 (m, 4).

Anal. Calcd for $C_5H_{10}N_2OS_2$: C, 33.69; H, 5.65; N, 15.71; S, 35.97. Found: C, 33.90; H, 5.73; N, 15.57; S, 36.17.

Reaction of Lead(II) Acetate Trihydrate with 2-Mercaptoethanol and 2,9-Diaza-5,6-dithiacyclononanone. Lead(II) acetate (7.2 g, 0.019 mol) and 2,9-diaza-5,6-dithiacyclononanone (3.38 g, 0.019 mol) were added to 350 ml of doubly distilled water and the mixture flushed with argon. 2-Mercaptoethanol (2.66 ml, 0.038 mol) was added by syringe, and the mixture stirred at room temperature for 12 h and refluxed under argon for 7 h. The flask was cooled to 0 °C, and the product filtered and washed with chloroform, to give 6.2 g (85%) of lead(II) 2,2′-dithiodiethylurea as a yellow-orange powder: mp 220 °C

dec; ir (Nujol) 3320, 1620, 1270, 1220, and 625 cm^{-1}; NMR (CF_3CO_2H) δ 2.8 (t, 4, J = 6 Hz) and 3.6 (t, 4, J = 6 Hz).

***N,N'*-Bis(2-mercaptoethyl)urea.** A suspension of lead(II) 2,2'-dithiodiethylurea (3.95 g, 10.25 mmol) in 300 ml of $CHCl_3$ was flushed with argon, and H_2S was bubbled through the suspension for 5 min, during which time the reaction mixture became black. The suspension was flushed with argon for 0.5 h to remove excess H_2S from the solution and filtered under argon through a Celite pad. The solvent was removed under reduced pressure and the pressure restored to 760 mm using nitrogen. The product, a white solid obtained in 94% yield (1.73 g), showed mp 118–119.5 °C; ir (Nujol) 3325, 3140, 1620, 1595, 1275, 1240, 1205, and 665 cm^{-1}; NMR ($CDCl_3$) δ 1.35 (t, 2, J = 8 Hz, SH), 2.45–2.9 (m, 4, $HSCH_2$), 3.4 (q, 4, J = 6 Hz, $NHCH_2$), and 5.05 (br, 2, NH); mass spectrum (20 eV) m/e M^+ 180.

Anal. Calcd for $C_5H_{12}N_2OS_2$: C, 33.31; H, 6.71; N, 15.54; S, 35.57. Found: C, 33.54; H, 6.48; N, 15.29; S, 35.47.

Thioglycolic Acid Hydrazide. Distilled thioglycolic acid methyl ester (20 ml, 23.06 g, 0.2173 mol) was transferred by syringe into a 500-ml round-bottomed flask which had been flame dried under argon and charged with 250 ml of dry, argon-saturated methanol. To this mixture was added 3.2 ml (0.095 mol) of 95% hydrazine (anhydrous). The reaction mixture was refluxed under argon for 24 h and cooled to room temperature, and the solvent removed under reduced pressure. The resulting solid product was filtered in a glove bag under argon, washed with dry, deaerated ethyl acetate, and dried at 0.01 mm and room temperature for several days. The hygroscopic product, obtained in essentially quantitative yield, had mp 50–52 °C (lit.[19] mp 50–52 °C); ir (Nujol) 3170, 3030, 2920, 2850, 1590, 1490, and 1150 cm^{-1}.

Anal. Calcd for $C_2H_6N_2OS$: C, 22.63; H, 5.70; N, 26.39; S, 30.21. Found, C, 22.73; H, 5.87; N, 25.85; S, 30.60.

Determinations of the extinction coefficient for Ellman's anion, $EllS^-$ were carried out under argon using solutions originally ca. 10^{-4} M in Ellman's reagent and ca. 10^{-3} M in 2-mercaptoethanol: we assumed that the tenfold excess of reducing agent would convert the Ellman's reagent to Ellman's anion quantitatively. Measurements were made carefully at the extinction maximum for the anion: λ_{max} 412 nm. At this frequency absorption due to Ellman's reagent is negligible.[4a,20] Neither 2-mercaptoethanol nor 2,2'-dihydroxydiethyl disulfide absorb at this wavelength. These values of the extinction coefficient were determined (ϵ, pH, buffer, buffer concentration M): 15 000, 9.0, TEA, 0.2; 14 800, 8.0, TEA, 0.2; 14 700, 7.0, TEA, 0.2; 14 600, 5.8, TEA, 0.2; 13 700, 7.0, phosphte, 0.05. This last value is in satisfactory agreement with previous determinations: 13 600, 7.0, phosphate, 0.25;[11c] 13 600, 6.5, phosphate, 0.133.[21] Throughout the work described in this paper, we have used ϵ 13 700. Ellman's reagent (pK_a = 4.75)[11a] is essentially completely ionized in the buffer systems used.

Quantitative Determination of the Rate of Decomposition of Ellman's Reagent. The stoichiometry of the decomposition of Ellman's reagent in base has been determined to be that shown in eq 22.[11c]

$$2EllS\text{-}SEll + 4OH^- \rightarrow 3EllS^- + EllSO_2^- + 2H_2O \qquad (22)$$

This decomposition was followed by measuring the increase in absorbance with time at 412 nm, due to $EllS^-$, in degassed solutions at 30 °C (0.2 M TEA buffers). Reactions were followed over 30 h. Initial slopes [$d(EllS^-)/dt$, M h^{-1}] for solutions initially 9.6×10^{-5} M were 1.38×10^{-6}, pH 9.0; 6.03×10^{-7}, pH 8.0; 1.42×10^{-7}, pH 7.0; 8.88×10^{-8}, pH 5.8. These correspond to decomposition of approximately these percentages of the original Ellman's reagent in 1 h: 1.0%, pH 9.0; 0.4%, pH 8.0; 0.1%, pH 7.0; 0.06%, pH 5.8. These estimates are of the same order of magnitude as reported previously.[11b]

Kinetics of Reduction of Ellman's Reagent by Thiols. One representative kinetic run will be described; others followed similar procedures. Dithiothreitol (DTT, 0.0773 g, 0.501 mmol) was transferred to a 100-ml volumetric flask which had been rinsed with pH 7.0 phosphate buffer solution and flushed with argon. The DTT solution was made up to volume, the volumetric flask stoppered, and the flask flushed with argon for 5 min and mixed by shaking. The stopper was removed and 1.0 ml of the solution was transferred to a 100-ml volumetric flask using an Eppendorf pipet. The solution was made up to volume in a similar manner to give a 0.501×10^{-4} M solution. Five milliliters of this latter solution was transferred to a 50-ml volumetric flask and made up to volume to give a 0.501×10^{-5} M solution. This solution was equilibrated in the 30 °C constant temperature bath connected to the cell compartment of the uv spectrophotometer. The same techniques were used to prepare solutions of Ellman's reagent with concentrations 1.0×10^{-3}, 0.5045×10^{-4}, and 0.5045×10^{-5} M. All solutions were kept under a positive pressure of argon.

Four 1-cm uv cells, fitted with serum stoppers, were flushed with argon. Two milliliters of the 10^{-4} M Ellman's reagent solution was transferred to a cell using a micrometer syringe. The cell was restoppered, equilibrated to 30 °C, and placed in the cell compartment. The spectrometer was zeroed. Two milliliters of the 10^{-4} M DTT solution was drawn into the micrometer syringe, the needle was pushed through the serum stopper of the cell, the recorder was turned on, and the reagent was added as quickly as possible. The reaction was followed by observing the change in absorbance of Ellman's anion at 412 nm; the reaction temperature was recorded before and after the experiment by placing a small EXAX thermometer (14–36 °C) inside the cell compartment. Control experiments included runs in solutions containing DTT but no Ellman's reagent and vice versa, to check for baseline drift. A run containing a ten-fold higher concentration of Ellman's reagent oxidized all of the DTT, and served to check that the starting concentration of DTT calculated from the weight was the same as that found spectrophotometrically. An experiment involving a ten-fold increased concentration of DTT served the same purpose for the Ellman's reagent.

Representative kinetic data are shown in Figure 1. Analysis of these data followed the procedure outlined in the text, and was accomplished by a computer program written for that purpose.[22]

Uv Light Has No Influence on the Thiol–Disulfide Interchange Reaction. Two cells containing Ellman's reagent were prepared at the same time. The cells were equilibrated in the 30 °C bath and placed in the cell compartment. Thioglycolic acid was added to the first cell, and a stopwatch was turned on; as the acid was added to the second cell the stopwatch time was noted and the recorder turned on. Since the solutions were dilute, the reaction was sufficiently slow that inaccuracies caused by this clumsy method of starting the reaction were minor. One of the uv cells was kept almost continuously in the uv light path and the other was placed in the beam only infrequently to take the absorbance readings. Because thioglycolic acid reacts more slowly with Ellman's reagent than do most of the other thiol reagents tested, and because low initial reactant concentrations were used to slow the reaction still further, the reaction was slower than any other reaction with Ellman's reagent in this study. No effect due to the spectrometer uv light was seen. In a separate experiment Ellman's reagent (0.0040 g, 1.0×10^{-5} mol) was transferred to a 100-ml volumetric flask and the solution made up with a deaerated phosphate buffer containing 10^{-6} M EDTA at pH 7.0. A uv scan from 310 to 465 nm was taken to show that no Ellman's anion was present. The volumetric flask was closed and placed in a Rayonet reactor, and illuminated using nine 350-nm and seven 253.7-nm lamps for 3 days. At the end of this time, the contents of the flask were deep yellow and the flask was warm to the touch. A second uv scan from 310 to 465 nm showed that 34% of the Ellman's reagent had been hydrolyzed, and that a quantity of Ellman's anion had formed which was consistent with hydroxide ion reaction as the cause of disappearance of the Ellman's reagent. Since the stoichiometry of the reaction was compatible with this reaction and since the quantity of Ellman's reagent consumed was approximately that expected at this pH, we conclude that light has no major effect on the rate of this reaction.

Influence of Buffer Composition and Concentration on k_1^{obsd} for Reaction of 2-Mercaptoethanol with Ellman's Reagent. Reactions were carried out using standard conditions in phosphate and TEA buffers at pH 7.0 [buffer, buffer concentration (M), k_1^{obsd} $\times 10^{-2}$ M^{-1} s^{-1}): phosphate, 0.05, 6.59; phosphate, 0.10, 8.00; phosphate, 0.20, 9.47; TEA, 0.05, 5.01; TEA, 0.10, 6.26; TEA, 0.20, 7.51. The ionic strength was held constant at 0.2 M in these reactions with KCl.

Determination of Thiol pK_a Values. Since the pK_a values of the dithiols differed by less than 2 units, their titration curves overlapped. Titration curves were obtained under argon with careful exclusion of atmospheric carbon dioxide, using 0.143 M carbonate-free potassium hydroxide solution.[23] Dilute solutions of thiols were used (0.001 M), so that activity corrections were not required. The KOH required to neutralize the thiol was added in 20 equal portions, and the pH of the solution measured 1 min after each addition. The pH meter was standardized against pH 7.00 and 10.00 Mallinckrodt BuffAR solutions. Analysis of the data followed a literature procedure,[24] using a computer program written for that purpose.[22]

Registry No.—Ellman's reagent, 69-78-3; thiolacetic acid, 507-09-5; 1,2:3,4-diepoxybutane, 1464-53-5; *threo*-1,4-dimercaptoacetyl-2,3-butanediol, 3483-12-3; *erythro*-1,4-dimercaptoacetyl-2,3-butanediol, 6892-68-8; 2,9-diaza-5,6-dithiacyclononanone, 60633-87-6; urea, 57-13-6; cystamine 2 HCl, 56-17-7; lead acetate,

301-04-2; 2-mercaptoethanol, 60-24-2; lead 2,2′-dithiodiethylurea, 60633-88-7; thioglycolic acid hydrazide, 760-30-5; thioglycolic acid methyl ester, 2365-48-2.

References and Notes

(1) Supported by the National Science Foundation (RANN, Grant G1 3428). Abbreviations used are: DTT, dithiothreitol; DTE, dithioerythreitol; EllS-SEll, Ellman's reagent, 5,5′-dithiobis(2-nitrobenzoic acid); EllS⁻, "Ellman's anion", the conjugate dianion of 2-nitro-5-thiobenzoic acid; CysSH, cysteine (or the cysteine moiety of a protein).
(2) M. Friedman, "The Chemistry and Biochemistry of the Sulfhydryl Group", 1st ed, Pergamon Press, Elmsford, N.Y., 1973; P. C. Jocelyn, "Biochemistry of the SH Group", Academic Press, New York, N.Y., 1972; Yu. M. Torchinskii, "Sulfhydryl and Disulfide Groups of Proteins", Plenum Press, New York, N.Y., 1974.
(3) W. W. Cleland, *Biochemistry*, **3**, 480 (1964); W. Konigsberg, *Methods Enzymol.*, **25**, 185 (1972).
(4) (a) G. L. Ellman, *Arch. Biochem. Biophys.*, **82**, 70 (1959); (b) A. F. S. A. Habeeb, *Methods Enzymol.*, **25**, 457 (1972).
(5) R. M. Evans, J. B. Fraser, and L. N. Owen, *J. Chem. Soc.*, 248 (1949).
(6) L. W. C. Miles and L. N. Owen, *J. Chem. Soc.*, 817 (1952); J. S. Harding and L. N. Owen, *ibid.*, 1528 (1954); L. Goodman, A. Benitez, and B. R. Baker, *J. Am. Chem. Soc.*, **80**, 1680 (1958).
(7) W. F. Beech, *J. Chem. Soc.*, 2483 (1951).
(8) J. P. Claudelli, German Patent 2 209 458; *Chem. Abstr.*, **77**, 164016 (1972).
(9) V. W. Cook, U.S. Patent 3 275 662; *Chem. Abstr.*, **66**, 16840a (1967).
(10) L. Schotte, *Ark. Kemi*, **9**, 377 (1956); D. J. Martin and C. C. Greco, *J. Org. Chem.*, **33**, 1275 (1968); J. P. Danehy, *Mech. React. Sulfur Compd.*, **2**, 69 (1967); F. Lautenschlaeger and R. T. Woodhams, "High Polymers", Vol. 26, K. D. Frisch, Ed., Wiley-Interscience, New York, N.Y., 1972, p 341; T. Taguchi, Y. Kiyoshima, O. Komori, and M. Mori, *Tetrahedron Lett.*, 3631 (1969); E. L. Eliel, V. S. Rao, S. Smith, and R. O. Hutchins, *J. Org. Chem.*, **40**, 524 (1975).
(11) (a) J. P. Danehy, V. J. Elia, and C. J. Lavelle, *J. Org. Chem.*, **36**, 1003 (1971); (b) J. P. Danehy and K. N. Parmeswaran, *ibid.*, **33**, 568 (1968); (c) J. P. Danehy, *Int. J. Sulfur Chem., Part B*, **6**, 103 (1971); (d) D. R. Hogg and P. W. Vipond, *Int. J. Sulfur Chem., Part C*, **6**, 17 (1971).
(12) A. Hanaki and H. Kamide, *Chem. Pharm. Bull.*, **19**, 1006 (1971); T. I. Kalman and T. J. Bardos, *J. Am. Chem. Soc.*, **89**, 1171 (1967); C. J. Swan and D. L. Trimm in "Oxidation of Organic Compounds", *Adv. Chem. Ser.*, **No. 76**, 182 (1968); C. F. Cullis and D. L. Trimm, *Discuss. Faraday Soc.*, **46**, 144 (1968).
(13) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism", 2d ed, Wiley, New York, N.Y., 1961.
(14) A. Schoberl and H. Grafje, *Justus Liebigs Ann. Chem.*, **614**, 66 (1958); G. Bergson and L. Schotte, *Acta Chem. Scand.*, **12**, 367 (1958); *Ark. Kemi*, **13**, 43 (1958).
(15) T. Serkine, K. A. Kato, K. Takamori, M. Machida, and Y. Kanaoka, *Biochim. Biophys. Acta*, **354**, 139 (1974).
(16) J. W. Ogilvie, J. T. Tildon, and B. S. Strauch, *Biochemistry*, **3**, 754 (1964).
(17) J. P. Danehy and C. J. Noel, *J. Am. Chem. Soc.*, **82**, 2511 (1960).
(18) D. M. E. Ruben and T. C. Bruice, *J. Am. Chem. Soc.*, **98**, 114 (1976).
(19) E. R. Atkinson, G. R. Handrick, R. J. Bruni, and F. E. Granchelli, *J. Med. Chem.*, **8**, 29 (1965).
(20) J. W. Donovan and T. M. White, *Biochemistry*, **10**, 32 (1971).
(21) H. Wenck and F. Schneider, *Experientia*, **27**, 20 (1971).
(22) J. E. Lilburn, Ph.D. Thesis, Massachusetts Institute of Technology, Cambridge, Mass., 1976.
(23) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases", Methuen, London, 1962, p 52.
(24) H. T. S. Britton, "Hydrogen Ions", 4th ed, Chapman and Hall, London, 1954, p 217 ff.

A Simple, Empirical Function Describing the Reaction Profile, and Some Applications

W. J. le Noble,*[1a] A. R. Miller,*[1b] and S. D. Hamann*[1c]

Department of Chemistry, State University of New York at Stony Brook, Stony Brook, New York 11794, and CSIRO, Applied Chemistry Laboratories, P.O. Box 4331, Melbourne, Victoria 3001, Australia

Received June 16, 1976

A simple, algebraic function is derived to represent the reaction profile of a concerted (one-step) reaction: $E = ax^4 + bx^3 - (4a + 3b)x^2/2$, where x is the reaction coordinate. It is shown that this function is in accord with the Hammond postulate and the Polanyi principle. It is used to evaluate the magnitude of the pressure-induced shifts of the transition state predicted by Walling several years ago, and to question the validity of a recent claim of the experimental verification of this effect. Further examination of this pressure effect leads to additional possibilities; among these are the vanishing of activation energies, the creation of certain new intermediates, and the conversion of degenerate sets of rapidly equilibrating structures into resonance hybrids.

The reaction profile showing how the energy of reacting molecules varies as they traverse the reaction coordinate has become a popular pedagogical device. The reason for this is that by means of it, one can conveniently illustrate a multitude of mechanistic phenomena. Concerted vs. stepwise reactions, intermediates vs. transition states, consecutive vs. competing reactions, early vs. late transition states, reversible vs. irreversible reactions, all these can be instantly indicated by means of the familiar curves one finds wherever mechanistically inclined chemists communicate with one another.

On a recent occasion we wished to make a quantitative estimate of pressure induced shifts of the transition state (vide infra), and discovered that none of the books exhibiting these curves records a function representing them.[2] We wish to describe an empirical function here for a simple, single-step reaction. We note its utility by showing, for example, that it behaves in the fashion demanded by the Hammond postulate and the Polanyi principle, and finally employ it to make the estimate referred to above.

The Function and Some of Its Features. We begin by noting that the general quartic

$$E = ax^4 + bx^3 + cx^2 + dx + e$$

is the simplest algebraic function which can have the general features of the reaction profile: a maximum flanked by two minima. E is the potential energy, and we let x represent the "distance" along the reaction coordinate, expressed as a fraction of the total to be traversed between the initial and final states. If we specify that at extreme values of x, E must be positive ($a > 0$), that the curve must pass through the origin ($e = 0$), that it must have a minimum there ($d = 0$) and at $x = 1$ [$c = -(4a + 3b)/2$], we have as the basic function

$$E = ax^4 + bx^3 - \frac{4a + 3b}{2}x^2 \qquad (1)$$

Several possibilities are shown in Figure 1; these include reactions with equilibrium constants less than, equal to, or greater than one (curves II, III, and IV, respectively, if we ignore the difference between energy and free energy), as well as extreme cases I and V which are reactions without activation energy. Beside the extrema at $x = 0$ and $x = 1$ (these points will be denoted by x_0 and x_1, respectively), there is a third (at $x^{\ddagger}$) which represents the transition state; it is found by dividing

$$\frac{dE}{dx} = 4ax^3 + 3bx^2 - (4a + 3b)x = 0$$